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<p>(21) International Application Number: PCT/US97/03313</p> <p>(22) International Filing Date: 27 February 1997 (27.02.97)</p> <p>(30) Priority Data:</p> <table border="0"><tr><td>60/012,705</td><td>28 February 1996 (28.02.96)</td><td>US</td></tr><tr><td>60/013,612</td><td>28 February 1996 (28.02.96)</td><td>US</td></tr><tr><td>60/020,003</td><td>21 June 1996 (21.06.96)</td><td>US</td></tr></table> <p>(71) Applicant (for all designated States except US): NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): VOLRATH, Sandra, L. [US/US]; 4225 Pine Oak Drive, Durham, NC 27707 (US). JOHNSON, Marie, A. [US/US]; 408 Heather Drive, Raleigh, NC 27606 (US). POTTER, Sharon, L. [US/US]; 3837 Whispering Branch Road, Raleigh, NC 27613 (US). WARD, Eric, R. [US/US]; 3003 Montgomery Street, Durham, NC 27705 (US). HEIFETZ, Peter, B. [US/US]; 3916 Sturbridge Drive, Durham, NC 27713 (US).</p> <p>(74) Agent: MEIGS, J., Timothy; 520 White Plains Road, Tarrytown, NY 10591-9005 (US).</p>		60/012,705	28 February 1996 (28.02.96)	US	60/013,612	28 February 1996 (28.02.96)	US	60/020,003	21 June 1996 (21.06.96)	US	<p>(81) Designated States: AU, BA, BB, BG, BR, BY, CA, CN, CU, CZ, FI, GE, GH, HU, JP, KG, KR, KZ, LC, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, TJ, UA, US, UZ, VN, YU, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published</p> <p><i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
60/012,705	28 February 1996 (28.02.96)	US									
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60/020,003	21 June 1996 (21.06.96)	US									
(54) Title: DNA MOLECULES ENCODING PLANT PROTOPORPHYRINOGEN OXIDASE AND INHIBITOR-RESISTANT MUTANTS THEREOF											

(57) Abstract

The present invention provides novel DNA sequences coding for plant protoporphyrinogen oxidase (protop) enzymes from soybean, wheat, cotton, sugar beet, grape, rice and sorghum. In addition, the present invention teaches modified forms of protop enzymes that are herbicide tolerant. Plants expressing herbicide tolerant protop enzymes taught herein are also provided. These plants may be engineered for resistance to protop inhibitors via mutation of the native protop gene to a resistant form or they may be transformed with a gene encoding an inhibitor-resistant form of a plant protop enzyme.

Soybean Protox-1, in the pBluescript SK vector, was deposited December 15, 1995 as pWDC-12 (NRRL #B-21516).

An alignment of the predicted amino acid sequences of the respective proteins encoded by the sequences shown in SEQ ID NOS: 2, 6, 10, 12, 15, 17, 19, 21, 23 and are set forth in Table 1. An alignment of the predicted amino acid sequences of the respective proteins encoded by the sequences shown in SEQ ID NOS: 4 and 8 are set forth in Table 2.

TABLE 1

Comparison of Protox-1 Amino Acid Sequences from Arabidopsis ("Arabpt-1"; SEQ ID NO:2), Maize ("Mzpt-1"; SEQ ID NO:6), Wheat ("Wtpt-1"; SEQ ID NO:10), Soybean ("Soybeanpt-1"; SEQ ID NO:12), Cotton ("Cottonpt-1"; SEQ ID NO:16), Sugar beet ("Sugpt-1"; SEQ ID NO:18), Rape ("Rapept-1"; SEQ ID NO:20), Rice ("Ricept-1"; SEQ ID NO:22), and Sorghum ("Sorghumpt-1"; SEQ ID NO:24)

Alignment is performed using the PileUp program (GCG package, University of Wisconsin, Madison, WI). Positions that may be modified according to the teachings herein to confer or enhance inhibitor resistance are shown in bold type.

	1		50
Rapept-1	MDLSLLRP.. QFFLSPPFSNP FPRSRYKPL	
Arabpt-1	MELSLLRPTT QSLLPSPFSKP NLRLNVYKPL	
Sorghumpt-1	
Mzpt-1	
Wtpt-1M ATATVAAASP LRGRVTGRPH	
Ricept-1	
Cottonpt-1MTAL IDLSLLRSP SVSPFSIPHH QHPRFRKPF	
Soybeanpt1MV SVFNEILFPP NQTLRLPSLH SPTSFTTSPT RKPPRSRPNP	
Sugpt-1	MKSMALSNCI POTQCMPLRS SGHYRGNCIM LSIPCSLIGR RGYYSKKRR	
	51		100
Rapept-1	NLRCSVSGGS WGSSTIEGG GGGRTVTADC	GXGXXG VIVGGGISGL CIAQALVTKH	
Arabpt-1	RLRCSVAGGP TVGSSKIEGG GGT.TITTDG	VIVGGGISGL CIAQALATKH	
Sorghumpt-1	
Mzpt-1ADC VVGGGISGL CTAQALATRH	



Wtpt-1 RVRPRCATAS SATETPAAPG VRL...SABC VIVGAGISGL CIAQALATRY
 Ricept-1
 Cottonpt-1 KLRCSLAEGP TISSSKIDGG ESS...IADC VIVGGGISGL CIAQALATKH
 Soybeanpt1 ILRCSLAEEES TASPPIR.. DSA...PVDC VVGGGVSGS CIAQALATKH
 Sugpt-1 MSMSCSTSSG SKSAVKEAGS GSGAGGLDC VIVGGGISGL CIAQALCTKH
 4x4x4x4

101 150

Rapept-1 PDA..AKNVM VTEAKDRVGG NIIT..REEQ GFLWEEGPNs FQPSDPLTM
 Arabpt-1 PDA..APNLI VTEAKDRVGG NIIT..REEN GFLWEEGPNs FQPSDPLTM
 Sorghumpt-1STVERPEE GYLWEEGPNs FQPSDPLTM
 Mzpt-1 ..G..VGDVL VTEARARPGG NITTVERPDE GYLWEEGPNs FQPSDPLTM
 Wtpt-1 ..G..VSDLL VTEARDRPGG NITTVERPDE GYLWEEGPNs FQPSDPLTM
 Ricept-1
 Cottonpt-1 RDV..ASNVI VTEARDRVGG NITTVER..D GYLWEEGPNs FQPSDPLTM
 Soybeanpt1 ..A..NANVV VTEARDRVGG NITTVER..D GYLWEEGPNs FQPSDPLTM
 Sugpt-1 SSSSLSPNFI VTEAKDRVGG NITTVER..AD GYLWEEGPNs FQPSDPLTM

151 200

Rapept-1 VVDSGLKDDL VLGDPTAPRF VLWNGKLRPV PSKLTDLFFF DIMSIPGKIR
 Arabpt-1 VVDSGLKDDL VLGDPTAPRF VLWNGKLRPV PSKLTDLFFF DIMSIPGKIR
 Sorghumpt-1 AVDSGLKDDL VFGDENAPRF VLWNGKLRPV PSKPADLFFF DIMSIPGKIR
 Mzpt-1 AVDSGLKDDL VFGDENAPRF VLWNGKLRPV PSKPADLFFF DIMSIPGKIR
 Wtpt-1 AVDSGLKDDL VFGDENAPRF VLWNGKLRPV PSKPGDLFFF DIMSIPGKIR
 Ricept-1
 Cottonpt-1 AVDSGLKDDL VLGDENAPRF VLWNGKLRPV PSKPTDLFFF DIMSIPGKIR
 Soybeanpt1 VVDSGLKDEL VLGPDAPRF VLWNRKLRPV PSKLTDLFFF DIMSIPGKIR
 Sugpt-1 AVDSGLKDEL VLGPDAPRF VLWNRKLRPV PSSLTDLFFF DIMTIPGKIR

201 250

Rapept-1 AGFGAIGIRP SPFGREESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPSK
 Arabpt-1 AGFGAIGIRP SPFGREESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPSK
 Sorghumpt-1 AGLGALGIRP PAFGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
 Mzpt-1 AGLGALGIRP PPFGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
 Wtpt-1 AGLGALGIRP PPFGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
 Ricept-1
 Cottonpt-1 AGFGAIGIRP PPFGRYEEVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK

Soybeanpt1 AGFGALGIRP PPPGHEESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPSK
 Sugpt-1 AALGALGFRP SPPGHEESVE HFVRRNLGDE VFERLIEPFC SGVYAGDPAK

251

300

Rapept-1 LSMKAAFGKV WKLEENGSSI IGGAFKAIQA KKNAPKTTRD PRLPKPKGQT
 Arabpt-1 LSMKAAFGKV WKLEQNGSSI IGGTFKAIQE RKNAPKAERD PRLPKPQGQT
 Sorghumpt-1 LSMKAAFGKV WRLEEEAGSSI IGGTIKTIQE RGNPKPFRD PRLPKPKGQT
 Mzpt-1 LSMKAAFGKV WRLEETGGSI IGGTIKTIQE RSKNPKPPRD ARLPKPKGQT
 Wtpt-1 LSMKAAFGKV WRLEETGGSI IGGTIKAIQD KGNPKPFRD PRLPAPKGQT
 Ricept-1 RALKAAFGKV WRLEDIGSSI IGGTIKTIQE RGNPKPFRD PRLPTPKGQT
 Cottonpt-1 LSMKAAFGRV WKLEETGGSI IGGTFKTIQE RNKTPKPPRD PRLPKPKGQT
 Soybeanpt1 LSMKAAFGKV WKLEKNGSSI IGGTFKAIQE RKGASKPPRD PRLPKPKGQT
 Sugpt-1 LSMKAAFGKV WKLEQKGGSI IGGTLKAIQE RGSNPKPPRD QRLPKPKGQT

301

350

Rapept-1 VGSFRKGLTM LPEAISARLG DKVKVSWKLS SITKLASGEY SLTYETPEGI
 Arabpt-1 VGSFRKGLRM LPEAISARLG SKVKLSWKLS GITKLESGGY NLTYETPDGL
 Sorghumpt-1 VASFRKGLAM LENAITSISLG SKVKLSWKLT SMTKSDGKGY VLEYETPEGV
 Mzpt-1 VASFRKGLAM LENAITSISLG SKVKLSWKLT SITKSDKGY VLEYETPEGV
 Wtpt-1 VASFRKGLAM LENAISARLG SKVKLSWKLT SITKADNGY VLGYETPEGL
 Ricept-1 VASFRKGLTM LPDAITSRLG SKVKLSWKLT SITKSDNGY ALVYETPEGV
 Cottonpt-1 VGSFRKGLTM LPEAIANSISLG SNVKLSWKLS SITKLGNGGY NLTFETPEGM
 Soybeanpt1 VGSFRKGLTM LPDAISARLG NKVKLSWKLS SISKLDSGEY SLTYETPEGV
 Sugpt-1 VGSFRKGLVM LPTAISARLG SRVKLSWILS SIVKSLNGEY SLTYDTPDGL

351

400

Rapept-1 VIVQSKSVVM TVPSHVASSL LRPLSDSAE ALSKLYYPPV AAVSISYAKE
 Arabpt-1 VSVQSKSVVM TVPSHVASGL LRPLSESAAN ALSKLYYPPV AAVSISYPKE
 Sorghumpt-1 VLVQAKSVIM TIPSIVASDI LRPLSGDAAD VLSRFYYPV AAVIVSYYPE
 Mzpt-1 VSVQAKSVIM TIPSIVASNI LRPLSSDAAD ALSRFYYPV AAVTVSYYPE
 Wtpt-1 VSVQAKSVIM TIPSIVASDI LRPLSIDAAD ALSKFYYPV AAVTVSYYPE
 Ricept-1 VSVQAKTVVM TIPSIVASDI LRPLSSDAAD ALSIFYYPV AAVTVSYYPE
 Cottonpt-1 VSLQSRSVVM TIPSHVASNL LHPLSAAAAD ALSQFYYPV ASVTVSYYPE
 Soybeanpt1 VSLQCKTVVL TIPSIVASTL LRPLSAAAAD ALSKFYYPV AAVSISYPKE
 Sugpt-1 VSVRTKSVVM TVPSIVASRL LRPLSDSAAD SLSKFYYPV AAVSLSYYPE

401 450

Rapept-1 AIRSECLIDG ELKGFGQLHP RIQKVETLGT IYSSSLFFNR APPGRVLLIN
 Arabpt-1 AIRTECLIDG ELKGFGQLHP RIQGVETLGT IYSSSLFFNR APPGRILLIN
 Sorghumpt-1 AIRKECLIDG ELQGFQQLHP RSQGVETLGT IYSSSLFFNR APAGRVLLIN
 Mzpt-1 AIRKECLIDG ELQGFQQLHP RSQGVETLGT IYSSSLFFNR APDGRVLLIN
 Wtpt-1 AIRKECLIDG ELQGFQQLHP RSQGVETLGT IYSSSLFFNR APAGRVLLIN
 Ricept-1 AIRKECLIDG ELQGFQQLHP RSQGVETLGT IYSSSLFFNR APAGRVLLIN
 Cottonpt-1 AIRKECLIDG ELKGFGQLHP RSQGIETLGT IYSSSLFFNR APSGRVLLIN
 Soybeanpt1 AIRSECLIDG ELKGFGQLHP RSQGVETLGT IYSSSLFFNR APPGRVLLIN
 Sugpt-1 AIRSECLING ELQGFQQLHP RSQGVETLGT IYSSSLFFGR APPGRILLIS

451 500

Rapept-1 YIGGATNIGI LSKSEGELVE AVDRDLRKML IKPSSIDPLV LGVKLWQAI
 Arabpt-1 YIGGSINIGI LSKSEGELVE AVDRDLRKML IKPNSTDPLK LGVRVWQAI
 Sorghumpt-1 YIGGATNIGI VSKTESELVE AVDRDLRKML INPTAVDPLV LGVRVWQAI
 Mzpt-1 YIGGATNIGI VSKTESELVE AVDRDLRKML INSTAVDPLV LGVRVWQAI
 Wtpt-1 YIGGSINIGI VSKTESDLVG AVDRDLRKML INPRAADPLA LGVRVWQAI
 Ricept-1 YIGGSINIGI VSKTESELVE AVDRDLRKML INPRAVDPLV LGVRVWQAI
 Cottonpt-1 YIGGATNIGI LSKTEGELVE AVDRDLRKML INPNAKDPLV LGVRVWQAI
 Soybeanpt1 YIGGATNIGI LSKTIDSELVE TVDRDLRKIL INPNAQDPFV VGVRLWQAI
 Sugpt-1 YIGGAKNPGI LNKSKDELAK TVDKDLRML INPDAKLPRV LGVRVWQAI

501 550

Rapept-1 PQFLIGHIDL VDAAKASLSS SGHEGLFLGG NYVAGVALGR CVEGAYETAT
 Arabpt-1 PQFLVGHFDI LDTAKSSLTS SGYEGFLFGG NYVAGVALGR CVEGAYETAI
 Sorghumpt-1 PQFLVGHLDL LEAAKSALDQ GGYDGLFLGG NYVAGVALGR CIEGAYESAA
 Mzpt-1 PQFLVGHLDL LEAAKAALDR GGYDGLFLGG NYVAGVALGR CVEGAYESAS
 Wtpt-1 PQFLIGHILDR LAAAKSALGQ GGYDGLFLGG KYVAGVALGR CIEGAYESAS
 Ricept-1 PQFLIGHLDH LEAAKSALGK GGYDGLFLGG NYVAGVALGR CVEGAYESAS
 Cottonpt-1 PQFLVGHLDL LDSAKMALRD SGYHGLFLGG NYVSGVALGR CVEGAYEVAA
 Soybeanpt1 PQFLVGHLDL LDVAKASIRN TGVEGLFLGG NYVSGVALGR CVEGAYEVAA
 Sugpt-1 PQFSIGHFDL LDAAKAALTD TGVKGLFLGG NYVSGVALGR CIEGAYESAA

551 563

Rapept-1 QVNDFMSRYA YK*
 Arabpt-1 EVNNFMSRYA YK*

Comparison of the Arabidopsis (SEQ ID NO:4) and Maize (SEQ ID NO:8) Protox-2 Amino Acid Sequences

Protox-2.Pep x Mzprotox-2.Pep

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1 .....MASGAVAD.HQIEAVSGKRVAV 21
      .| |:|: .: |...:|||
1 MLALTASASSASSHPYRHASAHTRRPRLRAVLAMAGSDDPRAAPARSVAV 50
22 VGAGVSGLAAYKLKSRGLNVTVFADGRVGGKLR SVMQNGLIWDEGANT 71
   |||||...|:|. :|||...:|.|||:|. :. :|||
51 VGAGVSGLAAYRLRQSGVNVTVFEAADRAGGKIRTNSEGGFVWDEGANT 100
72 MTEAEPEVGSLLDDLGLREKQQFPISQKKRYIVRNGVPVMLPTNPIELVT 121
   |||:| |.:|:|||||. :|||:| ||.|||||:|. |.:|.:|.:|. :.
101 MTEGEWEASRLIDDLGLQDKQQYPNSQHKRYIVKDGAPALIPSDPISLMK 150
122 SSVLSTQSKFQILLEPFLWKK...KSSKVSDASAEESVSEFFQRHFGQE 167
   |||||.||:..:||||:| |. :|||:.. -|||:..| :|||. |
151 SSVLSTKSKIALFFEPFLYKKANTRNSGKVSEEHLSSEVSGF CERHFGRE 200

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